MEMORIAL ARTICLE

Erythropoietin: a story of a discovery with Polish contribution

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In 2008, the history of erythropoietin research was presented among the most important discoveries in the special American Society of Hematology Anniversary Brochure, *50 Years in Hematology: Research That Revolutionized Patient Care*. Erythropoietin (EPO), a 35-kDa glycoprotein, is the physiologically obligatory growth factor for erythroid development. Erythropoietin exerts its erythropoietic action by binding to the specific high-affinity cell surface receptor (EPOR) expressed on erythroid progenitor and precursor cells in the bone marrow.

In adults, EPO is predominantly produced in the kidney by peritubular cells. Renal EPO production is under the control of an oxygen-sensing mechanism involving transcriptional regulation by hypoxia-inducible factor. Plasma EPO levels are measurable by a clinically available enzyme-linked immunosorbent test.

But what is the story behind it? In the last decade of the 19th century and the first years of the 20th century, the hypoxia phenomenon began to be associated with the rate of red cell production. Although some hypotheses were formulated, for example, by Friedrich Miesner[1] and Paul Carnot,[2] no clear explanation for this relationship was then proposed. However, it should be noted that it was Carnot who came up with the idea that humoral regulation is responsible for the production of red blood cells and the active, still hypothetical, factor located in serum was named *hemopoietin* (*hémopoïétine*).

But it was not earlier than in the 1940s and then 1950s that the next major steps were taken on the experimental level, showing that when anemic serum was injected in healthy rabbits, the induction of new red cell production occurred within 3 to 6 days.[3] In 1943, Newton Krumdieck and Chaterine Walcott from Cornell University Medical College, New York, proved that anemic serum was triggering a "significant reticulocytosis" in the test animals.[4] Five years later, 2 Finnish researchers, Eva Bonsdorff and Eeva Jalavisto, working at the University of Helsinki, interested in the red blood cell production mechanism, named the hemopoietic substance *erythropoietin*.[5] In 1953, Danish-born Allan Erslev summarized the results of the experiments on animals and concluded: "Large amounts of plasma from rabbits, rendered anemic by bleeding, were injected into normal rabbits. A significant rise in the number of reticulocytes was observed in these rabbits."[6]

Simultaneously, more evidence demonstrated that the hypoxic stimulation of erythropoiesis was an effect of the indirect humoral mechanism.[3] Kurt Reissman, in an experimental model, used 2 parabiotic rats with direct connection of their blood circulatory systems at the capillary level. The first animal was kept in conditions of inducted hypoxia, while the other, in normal atmospheric environment. Both rats developed characteristic sings of reticulocytosis and increased hemoglobin. Bone marrow hyperplasia was also present. These results led Reissman to the conclusion that there must be some "humoral factor elicited by the hypoxemia in the one partner and transferred to the other one."[7] At the same time Gerhard Ruhenstroth-Bauer became seemingly convinced of the humoral regulation and published 2 papers in German in 1950 and 1952.[8,9]

That opened questions on the real nature of erythropoietin (EPO) and the possible place or places where it is produced. The first important reports were published in 1957. Leon Jacobson and his coworkers informed that bilaterally nephrectomized animals, subjected to bleeding, failed to

produce increased EPO.[10,11] In the same year, clinical observations concerning patients with anemia and chronic renal failure confirmed the blocking of EPO production.[11,12] In the years 1958 to 1959, a Norwegian, Sverre Osnes, experimenting on mice after their kidneys' irradiation and performing partial nephrectomy, became convinced that "the serum from nephritic mice with anemia can induce reticulocytosis in normal mice (erythropoietin effect)"[13] and that kidneys "may produce a principle of importance in erythropoiesis, and lack of this principle may be the cause of the anemia developing in x-irradiated nephritic mice."[14] Then, after further research in which he used different experimental methods with "the injections into mice of serum" both from animals and from "human beings with various types of anemia," new evidence showed that an important factor for erythropoiesis mechanism is produced in the kidney.[14] Finally, Osnes became convinced that the mechanism of EPO production was more complex, involving both the nervous and the hormonal system. The production of erythropoietic factors was to be connected with kidney and liver.[15] The renal localization of the EPO mechanism was also underlined by Gurney.[16]

In 1961, Fisher's team, using an isolated in situ perfused dog kidney, was able to clearly demonstrate enhanced production of EPO when cobalt was used as a stimulator. Not only did cobalt have a direct effect on erythropoiesis, but also a higher level of serum erythropoietin was present. Fisher admitted that the kidney was the probable source of EPO.[17,18]

In the same year a Polish researcher, Zofia Kuratowska (1931–1999; **FIGURE 1**) and coworkers published results of their study which provided a direct proof of this hypothesis.[19] They perfused, in a closed circuit, isolated rabbit organs: kidney, liver, lungs, and spleen with the animal's oxygenated or hypoxemic own blood. The possible erythropoietic activity of the extracts of this blood was tested in mice, the percentage of reticulocytes in the peripheral blood and examination of bone marrow smears were used as the indicators. As one of the co-authors of the paper, Bohdan Lewartowski, recalls "the idea of our research model was derived from the method of studying release of active bodies from isolated organs and tissues once used by my boss, Professor Franciszek Czubalski and called biodialysis."[20]

The researchers found that perfusates of hypoxic kidneys had clear erythropoietic activity, whereas there was none or negligible in those of oxygenated kidneys and oxygenated or hypoxic remaining organs. Therefore, Kuratowska concluded that kidneys could be "the main site of release of erythropoietin."[19] Nevertheless, in the discussion of the paper, she carefully considered the possibility that there might also be other sites of its release and that in the body, the kidney might be an important link in the complex mechanism of erythropoietin release in which the central nervous and endocrine systems might be involved.

In later 1960s, a probable formation of erythropoietin in perfused kidney models was investigated also by others.[21]

In the next step of investigation on erythropoietin by the Kuratowska's team, isolated kidneys were perfused with pure Tyrode solution in order to obtain renal factor separated from blood.[22] It was found that the hypoxic kidneys released a factor to the Tyrode solution that stimulated erythropoiesis in the experimental animals when injected intravenously, but its effect was negligible when administered subcutaneously. The renal factor became fully active when incubated with α fractions of plasma proteins. This led to a hypothesis that the renal factor with the proteins forms a complex which increases its stability and promotes its transport to the bone marrow. Other possibilities were also considered. The first, that the renal factor activates a precursor of EPO in the plasma. The second possibility was that the renal factor may be activated by some unknown mechanism connected with α-globulin fractions.

In 1968, Kuratowska, conducted a new set of experiments. As a result, she stated that the kidney releases a labile component which she named the renal erythropoietic factor. Then, the labile component forms an active and relatively stable complex with α-globulin which is present in the blood plasma. In fact, Kuratowska postulated to identify the described complex as erythropoietin, which circulates in the blood and has a direct effect on the blood-forming organs. The analysis of the liver led to a suggestion that it may be a storage site and plays an important role in erythropoietin catabolism.[23] The mechanisms involved in elimination and site of EPO degradation are still not completely understood. In vitro studies suggest that about 60% of EPO are resecreted, and 40% undergo degradation in the cells expressing the EPO receptor.[24,25]

It should be added that Kuratowska was among the pioneers of the clinical application of EPO in Poland. She administered erythropoietin to a patient with multiple myeloma accompanied by severe anemia. The treatment was successful.

Epoetin alfa was the first recombinant product approved by the Food and Drug Administration in 1989 for use in patients with chronic kidney disease, followed by its approval in 1993 in the hematology and oncology supportive care. Currently, there are 3 recombinant human erythropoietin (rhEPO) preparations available for clinical use: epoetin alfa, epoetin beta, and darbepoetin alfa.

The main indication for rhEPO in patients with anemia is to increase hemoglobin to the lowest concentration possible to diminish or eliminate transfusion requirements, and it should be administered at the minimum effective dose.

Food and Drug Administration–approved indications for rhEPO are: patients with anemia due to chronic kidney disease undergoing dialysis or not, those with low and intermediate grade 1 myelodysplastic syndrome, or HIV-infected patients. Treatment with rhEPO may also be considered in patients with anemia during myelosuppressive chemotherapy for hematological malignancies (eg, lymphoproliferative neoplasms: multiple myeloma, non-Hodgkin lymphoma, chronic lymphocytic leukemia), and for some solid tumors. All these precautions are undertaken to avoid or diminish possible adverse events, for example, arterial hypertension, venous thromboembolism, and tumor progression in cancer patients. Pure red cell aplasia, due to the development of anti-EPO antibodies, is very rare and described predominantly in patients with chronic kidney disease.

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